EXHIBIT 37

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Airborne contamination of wounds in joint replacement operations: the relationship to sepsis rates

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Summary: During operations for total joint replacement done in operating rooms with conventional ventilation the mean air contamination varied considerably among the 15 hospitals studied. The range was from 51 to as many as 539 bacteria-carrying particles per cubic metre. When the data from all the hospitals were grouped according to the mean level of bacterial airborne contamination, including operations done in control and in ultraclean air, there was a good correlation between the air contamination and the joint sepsis rate. There was also a correlation between the mean values of air contamination and the numbers of bacteria isolated from wound wash-out samples; but the apparent efficiency of the sampling method varied a great deal among the hospitals carrying out this procedure. From this data it would seem that by far the largest proportion of bacteria found in the wound after the prosthesis had been inserted reached it by the airborne route. With the mean air contamination found in the control series, 164 bacteria-carrying particles per cubic metre, this proportion was as much as 95 per cent.

The risk of joint sepsis varied widely among the 19 hospitals. The differences between the highest and lowest being probably as much as 20-fold. However, the effect of an ultraclean air environment was similar at all hospitals.

Introduction

In a previous paper we have presented the basic results of a randomized prospective study of the effects of ultraclean air in the operating room on joint sepsis after operations for total hip or knee joint replacement (Lidwell *et al.*, 1982). The data showed a substantial reduction of deep joint sepsis, confirmed at subsequent re-operation, when the insertion of the prosthesis was done in ultraclean air and the operating room staff wore conventional pattern clothing. When the scrubbed staff wore body-exhaust suits in an ultraclean air room, or when plastic isolators were used, the reduction in sepsis, compared with that after operations

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in conventionally ventilated operating rooms, was even greater. The perioperative prophylactic use of antibiotics was also associated with a considerable reduction in the incidence of sepsis.

In this paper we discuss, in more detail, the air contamination in the 19 hospitals taking part in the study in relation to the type of ventilation used; the relationship between airborne contamination, bacterial dose in the wound and sepsis; the numbers of bacteria isolated from wound wash-out samples and the variation between the different hospitals in the sepsis rates and the effect of ultraclean air. The list of the hospitals which took part is given in Lidwell *et al.* (1982).

Air contamination

Methods

To assess the exposure of the wound to airborne bacteria air samples were taken in the operating rooms between the time of first incision and final closure of the wound. For operations performed in ultraclean air there is no representative sampling position other than the vicinity of the wound itself. Surgical requirements restricted the positioning of the air sampling equipment but it was usually possible to take the sample from within 30 cm of the wound. At 14 hospitals Casella slit samplers were used which had been adapted in a way generally similar to that described by Lidwell, Richards and Polakoff (1967). At the five other hospitals the samples were taken through Sartorius gelatine-membrane filters and the holders were small enough to be placed close to the wound. When plastic isolators were used (Trexler, 1973) representative samples could be taken from the air discharge. At four hospitals petri dishes were exposed to give a direct estimate of the numbers of bacteria settling on an uncovered surface.

Conventional non-selective media were used and only aerobic cultures were usually made. The colonies were counted after incubation for 36 h at 37°C. At two hospitals samples were also incubated anaerobically; these were collected on gelatine-membrane filters, which give the best isolation of anaerobic species (Hambraeus and Bendiktsdóttir, 1980).

Only small numbers of colonies were grown from the samples taken in the cleanest air. It was therefore necessary to make allowance for possible contamination either of the medium itself or introduced during handling of the plate. To assess this effect some plates were taken into the operating room, placed in the sampler, removed without drawing any air over them and incubated.

Excessively prolonged sampling times and overlarge volumes of air drawn over the plate can lead to loss of viable organisms by surface drying. A similar loss may also occur on gelatine-membrane filters. For a 14 cm petri dish, 10 m^3 of air and a 10–15 min sampling time usually represent safe limits, but the results are affected by the ambient temperature and humidity.

Owing to the very large number of operations included in the study, air samples were taken only during a proportion of them. Approximately 20 air samples were taken at each hospital for each ventilation–clothing combination; this represented a sample of about 10 per cent.

Results

The logarithms of the values obtained from individual observations under one set of conditions at any one hospital conformed, as is usual for this type of observation, to a normal distribution. Geometric means were therefore calculated for each set and these are shown in Table I for each hospital.

The geometric mean of the mean values of air contamination, expressed as colony-forming units per cubic metre (cfu m^{-3}), of the 15 sets from operating rooms with normal turbulent ventilation and the staff wearing conventional pattern clothing, the control sets, was 164 m^{-3} (Lidwell *et al.*, 1982); the range was from 51 to 539 m^{-3} and the geometric standard deviation of the hospital mean values was 2·0. The variability between hospitals using similar ultraclean air systems was at least as great. For the six hospitals using a walled downflow system the mean value, when body-exhaust suits were worn, was 0·40 m^{-3} and the geometric standard deviation of the six values was 2·3, but the accuracy was low for the very small numbers of bacteria isolated from such very clean air. The number of hospitals employing any other ultraclean air systems was too small to justify any attempt to estimate the between-hospital variation. The variability of the individual samples at any one hospital was considerable; the geometric mean of the 14 estimates of the geometric standard deviation available from the control sets (no estimate was made for one hospital with only three observations) was 1·8. It was somewhat higher, about 2·5, for the six hospitals using walled downflow and body-exhaust suits. For a set of 20 observations under similar conditions the standard error of the means will then be 1·13, i.e. antilog[(log 1·8)/$\sqrt{20}$] for the controls and 1·23 for walled downflow.

The dummy plates showed a considerable variation at the different hospitals, the range being from 1·5 colonies per plate to less than one colony per 10 plates. The actual dummy values at each hospital were used to correct the estimate of air contamination. This correction was insignificant wherever the mean bacterial count exceeded 1 m^{-3} but did reach around 50 per cent at two hospitals with corrected levels of 0·15 and 0·3 m^{-3}. These high correction values reduce the accuracy of the estimates of the mean counts in the cleanest air group and account for at least some of the increased inter-hospital variability in this group compared with that for the control series.

The inter-hospital variation shown in the control series, a geometric standard deviation of 2·0, is greatly in excess of that from the intra-hospital sample variation, which results in a geometric standard deviation of the mean for a hospital of no more than 1·13 for the control sets, and a 95 per cent range of about 130–210. The reasons for the much larger differences observed between hospitals are of considerable interest and concern. They might be determined by differences relating to the persons involved or by differences in the operating room environment. The importance of restricting the numbers and activity of persons in the operating room has been emphasized by Blowers (1963). Although the number of scrubbed staff varied very little from hospital to hospital, with an average, when the anaesthetist is added, of about five, the numbers of unscrubbed staff present certainly differed considerably. There are undoubtedly great variations between

Table I. *Results from individual hospitals*

Hospital no.	Ventilation*	Type of clothing†	Air contamination, geometric mean value (CFU m^{-3})‡	Number of operations		Number of joints		Relative risk, R	Deviation, δi	Relative deviation, $\delta i/\sqrt{\Sigma i}$
				Total	With antibiotics	Septic, i	Expected septic, I			
01	C	C	396	72	3	2	2·78	0·88	−0·90	−0·52
	DW	BE	0·2	69	1	1	0·62			
02	C	C	380	102	2	1	4·39	0·19	0·31	0·31
	I	C	1·5	72	4	0	0·74			
03	C	C′	156	265	245	0	2·53			
	DW	C′	2·6	111	106	1	0·36	0·28	−1·42	−1·42
	DW	BE	0·3	170	133	0	0·66			
04	C	C	217	98	86	2	1·20	1·52	0·36	0·21
	H	C	70	87	72	1	0·78			
05	C	C	229	403	247	4	7·85	0·55	0·62	−0·28
	H	C	25	109	50	1	1·26			
06	C	C″	539	292	166	21	8·52	2·63	−2·86	−0·54
	DW	C″	3·8	318	190	7	2·11			
07	C	C	112	73	71	0	0·55	—	—	—
	I	C	0·5	28	27	0	0·08			
08	C	C‴	81	429	427	4	2·71			
	H	C‴	6·5	203	203	1	0·67	1·24	1·28	0·57
	H	BE	1·0	252	252	0	0·65			
09	C	C	123	141	126	0	1·32	—	—	—
	D	C	20	189	159	0	1·16			

10	C	C	140	168	23	1	4·53	0·50	−2·52	−1·45
	D	C	4·7	139	26	2	1·49			
11	C	C	131	315	133	11	6·30			
	D	C	12	68	24	1	0·74	1·51	2·99	0·83
	DW	BE	1·6	193	61	1	1·58			
12	C	C	74	619	617	3	3·76	0·53	2·00	1·15
	DW	BE	0·7	744	739	0	1·88			
13	C	C	142	148	73	0	2·77	—	—	—
	DW	C	1·1	217	121	0	1·31			
14	C	BE	51	141	141	1	0·75	0·86	0·71	0·71
	DW	BE	0·3	141	140	0	0·41			
15	C	C	332	236	14	8	9·40	0·95	−1·90	−0·57
	I	C	0·15	238	5	3	2·14			
16	A	C	25	67	36	1	0·70	0·97	0·64	0·64
	A	BE	4·5	48	28	0	0·33			
17	C	C	51	380	378	4	2·06			
	DW	C	1·9	163	162	2	0·46	2·30	−1·46	−0·93
	DW	BE	0·2	227	227	1	0·53			
18	A	C	89	72	72	0	0·46	1·43	−1·31	−1·31
	A	BE	38	44	43	1	0·24			
19	A	C	54	108	108	0	0·59	—	—	—
	A	BE	17	93	90	0	0·41			

* *Ventilation:* C=control, positive supply; H=horizontal air flow; D=downflow without walls below 7 ft (2·1 m); DW=downflow with walls down to near floor; A=Allander system, input through perforated ceiling over table enclosed by high-velocity air curtain; I=isolator.

† *Clothing:* C=conventional pattern cotton gowns; C′=disposal paper gowns also used; C″=gowns of a non-woven material; C‴=disposable gowns ('Vigilon'); BE=body exhaust.

‡ Membrane filtration used in hospitals 10 and 16–18.

individuals, and for the same individual at different times, in the rate at which bacteria are dispersed into the air from the skin, depending on the density of colonization of the skin surface and the rate at which skin squames are shed during moderate exercise (Hare, 1963; May and Pomeroy, 1973; Hill, Howell and Blowers, 1974; Noble *et al.*, 1976; Whyte, Vesley and Hodgson, 1976; Mackintosh *et al.*, 1978). The ventilation of the 'control' operating rooms, as recorded, varied from about 0·35 to 1·2 $m^3 s^{-1}$, but few of these values represented actual measurements and the often wide divergence between specified and achieved ventilation volumes is notorious (Abel, 1973). The effects of the differences on the levels of air contamination will be less than the ratio of the volumes, owing to loss of particles by settling, but are still likely to be considerable (Lidwell, Richards and Polakoff, 1967).

At the two hospitals (numbers 19 and 7) where samples were examined by anaerobic cultivation as well as aerobically, the mean numbers of colonies grown aerobically were similar to the numbers grown anaerobically in a CO_2-enriched atmosphere (Gas Pak). In the control operating rooms the figures were 50 and 114 m^{-3} on anaerobic cultivation compared with 54 and 112 m^{-3} on aerobic cultivation. In the ultraclean air situation the corresponding values were 13 and 1 m^{-3} as against 17 and 0·5 m^{-3}. The above figures for anaerobic cultivation include both facultative and obligate anaerobes; in this group of organisms *Propionibacterium* spp. were by far the most numerous. At both these hospitals, and three others indicated in Table I, gelatine-membrane filters were used. There is reason to suspect that the number of colonies isolated from a given volume of air after aerobic cultivation when using this method is less than would be found from the slit sampler, but there is no direct comparative evidence to substantiate this.

From settling plates and volumetric air samples taken in parallel it is possible to make an estimate of the settling velocity of the bacteria-carrying particles. At four hospitals this was done in the control situation. The estimated settling rates were very similar, namely 0·26, 0·19, 0·19 and 0·18 m min^{-1}, which are similar to those reported elsewhere (Lidwell, Richards and Polakoff, 1967; 0·21 m min^{-1}). For only the first two of the above hospitals, however, were there enough data to give a figure in the ultraclean situation; the estimated rates for this were 0·78 and 16 m min^{-1}. The higher values, especially the last, suggest that direct deposition of large particles may sometimes contribute disproportionately to settling. In the two hospitals where these results were obtained the volumetric air contamination in the ultraclean air was reduced 104-fold and nearly 2000-fold respectively compared with the control value, but the apparent reductions in the numbers settling were only 34-fold and 23-fold.

A similar difference has been found by some other workers, e.g. settling rates of 0·18 and 0·9 m min^{-1}, with reduction in the clean room of 230-fold volumetric and 50-fold settling (C. O. Bechtol, personal communication). Similar differences are also shown in the data collected by Lindberg (1979) and Nelson (1977).

The relationship between airborne contamination, bacterial dose in the wound and sepsis rate

Methods

Direct comparison between the incidence of sepsis for operations done in control conditions, with conventional positive pressure (turbulent) ventilation, and for those done in ultraclean air, where these were performed by the same surgeons allotting their patients at random to the different conditions, has shown substantial reductions associated with the use of ultraclean air. In an attempt to derive a relationship between the actual level of air contamination and the incidence of sepsis, the operations done in the 42 sets of conditions listed in Table I have been divided into six groups according to the actual mean levels of air contamination recorded. The groups no longer provide strictly controlled comparisons because surgeons and hospitals are differently combined within them. However, this lack of homogeneity will, to some extent, be counteracted by the averaging effect of the six to nine conditions included in each group. The characteristics of the groups are set out in Table II, and the mean incidence of

Table II. *Joint sepsis and air contamination level*

Group	Number of hospitals	Air contamination (bacteria-carrying particles m^{-3}) Mean (geometric)*	High	Low	Number of operations N_1	N_2	N'	Septic Number	%
I	6	345	539	217	685	518	814	38	4·67
II	6	136	156	112	439	671	607	12	1·98
III	7	68	89	51	21	1815	475	13	2·71
IV	6	21	38	12	168	402	268	4	1·49
V	9	2·9	6·5	1·1	563	901	788	14	1·78
VI	8	0·4	1·0	0·15	344	1519	724	5	0·69
All	42	—	—	—	2220	5826	3676	86	2·34

N_1, number operated without prophylactic antibiotics; N_2, number operated with prophylactic antibiotics; $N' = N_1 + N_2/4$ (effective risk units).

* Weighted for the values of N' at each hospital.

sepsis, corrected to that for patients not receiving prophylactic antibiotics, is shown in Figure 1 in relation to the level of air contamination. Overall, the incidence of joint sepsis among patients who received prophylactic antibiotics was one-quarter of that among those not so treated. The risk of sepsis in any group, for patients not receiving antibiotics, has therefore been estimated by dividing the total number of septic joints recorded in that group by the number of patients in the group who had not received prophylactic antibiotics plus one-quarter of the number of those who had.

Results

There is clearly a strong correlation between the level of air contamination and the incidence of sepsis. In Figure 1 air contamination has been shown on a

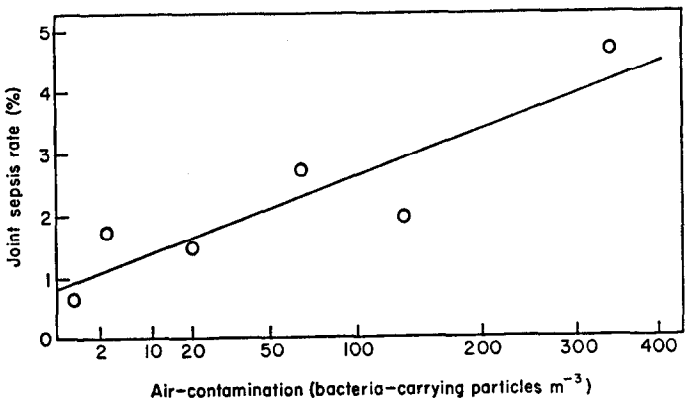

Figure 1. The relationship between mean air contamination and the incidence of joint sepsis. Each point is the mean of the data from between six and nine hospitals (see Table II). The straight line is the regression of sepsis rate on the square root of the air contamination.

square root scale, which gives a convenient expansion at lower values without loss of the zero. There is no theoretical justification for this function, but it provides a useful basis for interpolation, and studies of the incidence of nasal colonization with *Staphylococcus aureus* have shown that this varies approximately as the square root of the numbers of particles carrying this organism in the inhaled air (Lidwell, 1981).

The regression of sepsis on the air contamination level, the line shown on the figure, is given by sepsis rate, I (in per cent)$=0{\cdot}84+0{\cdot}18\sqrt{A}$, where A is the air contamination in bacteria carrying particles per cubic metre. The correlation coefficient is 0·90 ($P<0{\cdot}02$) and the standard error of the slope$\pm$0·044.

Figure 1 does not show the relation to bacterial dose because there is some contamination of the wound by non-airborne routes, corresponding to the intercept of 0·84. This value should not, however, be accepted without reservation as an estimate of the incidence of sepsis from non-airborne routes because it is an extrapolation and the value depends on the functional relationship assumed. It is somewhat, but not significantly, larger than the value, 0·69, obtained for the operations carried out at an air contamination level of 1 m^{-3} or less (Table II). Evidence will be presented in a later section that the bacterial contamination of the wound by non-airborne routes is equivalent to that from the air at a contamination level of about eight bacteria-carrying particles per cubic metre. If this is added to the mean level of air contamination in each of the six groups, we can arrive at a relationship between dose and the incidence of sepsis. By making use of the data of Whyte, Hodgson and Tinkler (1982) it is possible to convert the relative dose, in terms of the air contamination, into approximate estimates of the actual numbers of bacteria deposited into the wound. With an estimated wash-out efficiency of 28 per cent, these authors recovered an average of 105 cfu at an air-contamination level of 400 bacteria-carrying particles per cubic metre. This corresponds to 105/0·28=375 cfu; i.e. the dose, in colony forming

units, is numerically similar to the air-contamination level, in bacteria-carrying particles per cubic metre of air.

With the dose calculated in this way the relationships are shown in Figure 2, on a double log scale to facilitate comparison with the nasal acquisition data for *Staph. aureus* referred to earlier. It is clear that the general form of the two sets of data is similar.

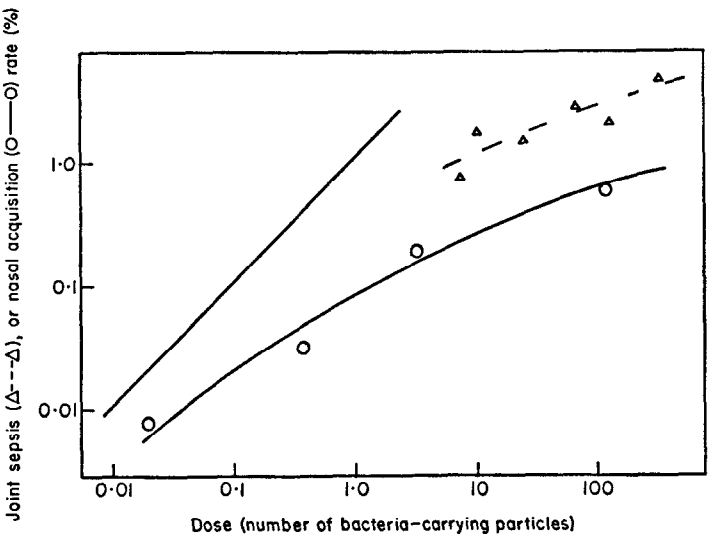

Figure 2. Bacterial dose and infection. Δ--Δ, joint sepsis rate; O——O, nasal acquisition of *Staph. aureus* (Lidwell, 1981). The straight line on the left of the figure is that for the sepsis (or nasal acquisition) rate which would result if 1 per cent of the particles deposited (or inhaled) led to sepsis (or nasal acquisition).

In both instances the dose, in numbers of viable bacterial cells, is probably several times larger than the number of airborne particles deposited or inhaled; in the case of the wound the values do not include the anaerobic species and in neither case has any allowance been made for the possibility of more than one viable cell per particle.

Bacterial contamination of the surgical wound during operation

Methods

After the insertion of the prosthesis but before closure of the wound was begun some wounds were sampled by washing out with fluid, usually quarter-strength Ringer solution. The liquid was injected into the wound cavity from a bladder syringe and either withdrawn back into the same syringe or, at some centres, if the patient was in the lateral position, allowed to flow out into a kidney dish held so that the lip of the dish was over the edge of the wound. Some 150 ml of solution were used in one or several injections and the recovered fluid was examined in one of two ways.

Table III. *Wound wash-out: data only from situations where more than 25 samples were obtained*

Hospital*	Operating room conditions†	Number of samples*	Geometric mean of counts (geometric S.D.)	Counts on dummy plates	C/UC‡	NAB/AB§	G/I‖	100α	*S*
06 (P)	C	65 (P)	120 (12)	1·6	—	2·7	—	—	—
	UC	73	3·5 (8)	—	34·3	1·5	—	21·8	2·7
13 (P)	C	33 (P)	21 (8)	0·6	—	—	—	—	—
	UC	48	1·4 (7)	—	15·0	4·2††	—	14·3	1·2
02 (P)	C	82 (P)	36 (9)	0·1	—	—	—	—	—
	UC:I	62	1·9 (6)	—	18·9	—	—	9·0	1·8
14 (P)	C	115 (P)	4·6 (7)	0·9	—	—	—	—	—
	UC:BE	113	0·38 (7)	—	12·1	—	—	8·3	0·4
03 (P)	C	261 (P)	12 (8)	0·5	—	—	4·7	—	—
	UC	102	1·5 (9)	—	8·0	—	—	6·8	1·3
	UC:BE	162	0·3 (14)	—	40·0	2·0	—	7·5	0·3
12 (P)	C	189 (P)	5·2 (10)	0·8	—	—	12·7	—	—
	UC:BE	288	1·4 (5)	—	3·7	—	6·1	5·2	1·4
09 (P)	C	147 (P)	7·8 (9)	(0·1)‡‡	—	—	—	—	—
	UC	161	2·8 (7)	—	2·8	—	—	4·9	1·8
19 (F)	C	112 (F)	2·0 (7)	0·3	—	—	—	—	—
	UC	120	1·0 (7)	—	2·0	—	—	2·7	0·5
10 (F)	C	118 (F)	4·8 (4)	2·2	—	—	—	—	—
	UC	96	2·8 (4)	—	1·7	—	—	1·5	2·7

11 (P)	C	228 (P)	1·8 (16)	2·3	—	5·0	6·4	—	—
	UC	41	1·1 (12)	—	1·6	—	—	0·6	1·0
	UC:BE	169	0·5 (24)	—	3·6	6·7	1·5	1·0	0·5
18 (F)	C	71 (F)	0·54 (6)	3·5	—	—	—	—	—
	UC	45	0·27 (5)	—	2·0	—	—	0·5	0·1
15 (P)	C	236 (P)	1·5 (9)	n.d.	—	—	—	—	—
	UC:I	236	0·47 (7)	—	3·2	—	—	0·3	0·5
01 (P)	C	70 (P)	1·5 (6)	n.d.	—	—	—	—	—
	UC:BE	68	0·5 (4)	—	3·0	—	—	0·3	0·5
07 (P)	C	56 (P)	0·32 (12)	0·2	—	—	—	—	—
	UC:I	31	0·30 (21)	—	1·1	—	—	0·02	0·3
Number	30	3598	See Table IV	11	16	5	5	16	16
Mean	—	120		0·74	5·0	3·2	5·1	—	—

* (P)=pour plates used; (F)=filter method used.

† C=control series; UC=ultraclean air (with conventional pattern clothing); UC:BE, body-exhaust suits worn; UC:I, plastic isolator used.

‡ C/UC=ratio of the geometric mean number of colonies isolated in the control series to that in the ultraclean air series.

§ NAB/AB=ratio of the geometric mean of the number of colonies isolated when antibiotics had not been used (NAB) to that when they had (AB).

‖ G/I=ratio of the geometric mean of the number of colonies isolated from those samples showing growth of the Oxford staphylococcus (G) on the plate to that from those samples where growth had been inhibited (I).

†† Only 18 samples on one side of the comparison.

‡‡ Same series as hospital 02.

α and S are defined in the text; a dash indicates that no appropriate entry is available or that there is insufficient data; n.d. indicates that a test was not done.

In method A the liquid was gently centrifuged to deposit unlysed blood and tissue fragments and the supernatant fluid was filtered under pressure through two membrane filters in series, with care taken to avoid transferring globules of fat. The equipment used was the Sartorius Sterilitest system (Sartorius, Göttingen), with a second filter holder attached to the top of the first. After all the fluid had passed through, the filters were washed with an additional 50 ml of sterile solution. The filters were then transferred to the surface of a blood agar plate for incubation. The deposit was taken up into a small volume of solution warmed to 50°C and added to an equal volume of double strength nutrient agar at 50°C. After thorough mixing the fluid was poured into a sufficient number of petri dishes to give pour plates in which the medium was reasonably translucent.

In method B, the wash-out fluid was added to about 200 ml of 1½ strength nutrient agar containing 0·1 per cent Tween 80, care being taken to see that neither specimen nor mixture reached a temperature above 55°C. To obtain thin translucent plates the fluid was poured into six to 10 petri dishes 140 mm in diameter. When possible the wash-out fluid was processed within 30 min; otherwise it was kept cool in a refrigerator or by immersion of the container in clean ice.

All plates were incubated at 37°C for more than 48 h, generally 3–4 days, to allow small slowly growing colonies to become visible. Colonies with the appearance of *Staph. aureus* were subcultured on phenolpthalein phosphate agar. Colonies showing a positive phosphatase reaction were examined for coagulase production. If prophylactic antibiotics had been used, pour plates were tested for inhibitory antibiotic by spotting with a drop of a broth culture of the Oxford strain of *Staph. aureus* (NCTC 6571). The filtration method removes antibiotic from the sample. It is also generally possible to neutralize any residual antibiotic in the sample, if necessary, by incorporating a wide spectrum β-lactamase. This was not usually necessary because, even when enough antibiotic was present to inhibit the Oxford staphylococcus, a sufficient proportion of the organisms still grew for comparison between the extent of bacterial contamination in wounds made in control or ultraclean air. The effect of inhibition (Table III) was much less than the variation in the efficiency of isolation of bacteria from the wounds at different hospitals.

Because the colonies found, particularly with samples from the ultraclean air environments, were often few and the numbers of plates used were considerable, dummy samples were processed in a similar way to the actual specimens. The use of a clean air cabinet reduced the risk of inadvertent contamination.

Results

Enough samples to enable reasonable estimates of the mean wound contamination, at least 25 at a given set of operating room conditions, were recorded from 14 hospitals. At two of these, samples were obtained from two different ultraclean air conditions. The summarized data from these hospitals are shown in Table III. Altogether 3598 samples were taken in 30 sets of operating-room conditions; i.e. the average number of samples for any one set of conditions was 120. At all

hospitals the mean number of colonies was less for operations done in ultraclean air conditions and, where more than one ultraclean air condition was in use at the same hospital, the number was least in the cleaner of the two. The colony counts were also less when prophylactic antibiotics had been given; this comparison could be made only in those hospitals where reasonable numbers of operations with or without the use of antibiotics had been sampled. The difference was greater when the specimens were classified according to the presence or absence of inhibition of the Oxford staphylococcus. Figure 3 illustrates some of these

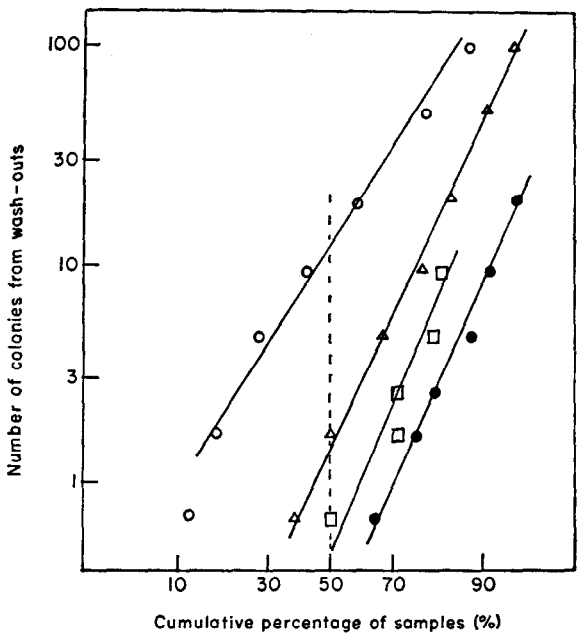

Figure 3. Distribution of colonies isolated from wound wash-out samples at hospital no. 03 (data from 508 samples). ○——○, Control conditions with prophylactic antibiotics (243 samples); △——△, ultraclean air with conventional operating room clothing and prophylactic antibiotics (103 samples); □——□, ultraclean air with body-exhaust suits but no prophylactic antibiotics (35 samples); ●——●, ultraclean air with body-exhaust suits and prophylactic antibiotics (127 samples).

points in relation to the results from one hospital and shows their general conformity to log–normal distributions. We have previously reported (Lidwell *et al.*, 1982) that, although there are very large differences in the apparent efficiency of isolation of bacteria from wounds by wash-out sampling at the different hospitals, the mean values are related to the average levels of air contamination.

Bacteria found in a sample may have been acquired from the air, may have been introduced into the wound in the operating room by non-airborne routes, or may be contaminants. This may be expressed as

$$W = S + aA,$$

Table IV. *Summary of wound wash-out samples*

Operating room conditions	Number of samples	Number of hospitals	Average number of samples	Air contamination (median cfu)	Wound wash-out median count (geometric S.D.)	Joint sepsis‡ (%)
Control	1793	14	127	142	4·5 (1·6)	1·5
Ultraclean air I*	686	8	86	7·3	1·5 (1·4)	0·9
Ultraclean air II†	1129	8	141	0·47	0·57 (1·3)	0·3
All	3598	30	120	—	—	—
Dummy samples	182	11	17	—	0·74 (1·4)	—

* Conventional pattern clothing worn.
† Body-exhaust suits worn or plastic isolators used.
‡ See Lidwell *et al.* (1982).

where W is the number found in the sample, A is the air contamination level and α a proportionality factor. We also have

$$S=D+K,$$

where K is the number by non-airborne routes and D that from contamination.

For the geometric mean values for W and A given in Table IV the regression of W on A is given by

$$W=0{\cdot}93+0{\cdot}025\ A.$$

Since D, from the same table, was 0·74, $K=0{\cdot}19$. This is equivalent to that deriving from the air at $A=0{\cdot}19/0{\cdot}025=7{\cdot}6$ m^{-3}.

The median value of A in the control series was 164 bacteria-carrying particles per cubic metre and at this value the contribution from the air would amount to $(0{\cdot}025\times164)/(0{\cdot}19+0{\cdot}025\times164)=96$ per cent.

The possible errors in this calculation are considerable but the substantial difference between the geometric mean of the control values and of those obtained in ultraclean air conditions leaves no doubt that by far the greater part of the bacterial contamination of wounds at operations done in the control operating rooms took place by the airborne route.

An alternative approach is to look at the individual results in Table III. If the variations in α are due to differences in the efficiency of isolating bacteria from the wound at the several hospitals, then the values of K might be expected to vary in the same way, i.e. K can be replaced by αa, where a is a constant, so that

$$S=D+\alpha a.$$

The regression of S on α for the 16 sets of values in Table III is given by

$$S=0{\cdot}60+9{\cdot}4\ \alpha.$$

The regression coefficient $r=0{\cdot}625$, $P\simeq0{\cdot}01$ and the standard error of the slope is $\pm3{\cdot}1$. The intercept of 0·60 does not differ significantly from the mean of the dummy samples, 0·74. At any value of α, $K=9{\cdot}4\ \alpha$ and the air contribution is $A\alpha$; i.e. the non-air contribution is equivalent to that deriving from the air at $A=9{\cdot}4$ m^{-3}. At the median value of A in the control series the contribution from the air will be $164/(9{\cdot}4+164)=95$ per cent.

These figures are very similar to those deduced by Whyte, Hodgson and Tinkler (1982) in a detailed study (some of their results are included in this study) which lead to an estimate of the non-airborne contribution equivalent to that from the air at a contamination level of 7·7 m^{-3}, and corresponding values for the airborne contribution in the control operating room.

If the reduction in the volumetric level of air contamination in ultraclean air is greater than that in the numbers settling, as has been suggested earlier, then the airborne contribution to the bacterial contamination of the wound will be even greater than that calculated above.

In an attempt to discover a procedural reason for the very wide differences in the number of bacteria recovered from the wound at the different hospitals a questionnaire was circulated enumerating 25 different aspects of the method.

When the replies were examined no clear reason for the differences emerged. Dividing the hospitals into two equal groups according to whether the value of α was above or below 0·04 showed no difference between the groups in respect of the degree of agitation, the volume of fluid recovered, or the use of β-lactamase. All the hospitals that used the filtration method were in the lower group, but some hospitals using the pour plate technique reported equally low values of α. All but one of the hospitals where Tween 80 was incorporated in the medium were in the upper group, but again there was a hospital in the lower group that also used Tween 80. The average period of incubation was longer in the upper group, about 5 days as against about 3 days in the lower group, but again there was overlap. The hospital with the lowest recovery rate of bacteria from its wounds used all the procedures that were otherwise associated with the higher values.

We have, therefore, no ascertained reasons for the wide variation in the apparent efficiency of the wash-out method in isolating bacteria from the wounds. The samples were, necessarily, taken by the individual surgeons at each hospital. The most plausible cause for the differences seems to be variation in the details of the technique used. It has been shown in animal experiments that variations in procedure can lead to large differences in the effectiveness of sampling (Harmer *et al.*, 1975). Better reproducibility has been claimed for a variant of the replica plating technique (Raahave, 1979) and further study of this might lead to improvements. Indeed subsequent work, using absorbent pads, at one of the centres which took part in this study and obtained rather low numbers in wound wash-out samples, only about one in 10 of the numbers in relation to the level of air contamination reported from the hospital with the highest proportionate recovery, has resulted in a more than 10-fold increase in the numbers of bacteria isolated from joint-replacement wounds (Benediktsdóttir and Hambraeus, 1983).

As would be expected, the mean numbers of bacteria isolated were fewer in those samples where there was enough antibiotic to inhibit the growth of the Oxford staphylococcus on the plates. The mean value of the ratio, based on five comparisons, was 5·1. A similar, but smaller, reduction (1/3·2) was observed when the wound had been rinsed with an antibiotic-containing solution or the patients had received prophylactic antibiotics, irrespective of whether this resulted in inhibition on the sample plates. The reduction in the mean numbers of bacteria found in wash-outs when operations were done in clean air (Table IV) parallels the reduction in the rates of joint sepsis. Similarly the reduction in the numbers of bacteria in the wash-outs associated with the use of antibiotics is similar to the 4:1 reduction in the incidence of sepsis among the patients who received prophylactic antibiotics (Lidwell *et al.*, 1982).

Variation between hospitals in the risk of sepsis and the effect of ultraclean air

Methods

From the interpolation formulae derived in preceding sections it is easy to cal-

culate the expected numbers of septic joints for each of the 42 sets of conditions (see Table I).

By combining the values at any one hospital the sum can be compared with the actual number of cases which occurred and a relative risk, R, calculated:

$$R=\sum i/\sum I,$$

where $\sum i$ is the actual number of cases of sepsis and $\sum I$ the calculated value.

It is also possible to derive, for each hospital, a figure for the expected difference between the number of instances of sepsis in the control series and the ultraclean air series corrected for the relative risk of sepsis (R) at the hospital and to subtract this from the observed difference, to arrive at a deviation from the expected difference:

$$\delta=i_c-\sum i_{uc}-R\,(I_c-\sum I_{uc}),$$

where the suffixes refer to the control and ultraclean air series respectively.

Because the variance of $(i_c-\sum i_{uc})$ is equal to $\sum i$, the values of $\delta i/\sqrt{\sum i}$ should be distributed about a mean of zero with unit standard deviation if there is no significant source of variation other than that due to the Poisson variance of the small numbers involved. This quantity, the relative deviation, therefore provides a useful way of examining the significance of the deviations observed at the individual hospitals.

Results

The values of R are shown in Table I. At five hospitals the number of instances of joint sepsis was no more than one and the calculated total $\sum I$ was little greater. For these hospitals no useful estimate of R can be made. The cumulative percentage distribution of R for the remaining 14 hospitals has been plotted in Figure 4. For two of these hospitals, which reported no instance of sepsis although the calculated values of $\sum I$ were 2·5 and 4 respectively, no more than upper limits for R can be estimated. Figure 4 only shows, therefore, actual values for those 11 hospitals where the estimated value of R exceeded the greater of these limits. The distribution of R follows an approximately log–normal form, with a median of 0·68 and a geometric standard deviation of 2·6. The method of calculation, whereby $\sum I=\sum i$, results in an arithmetic mean of unity. The variation of relative risk at the several hospitals, after allowing for differences in air contamination and antibiotic usage, is thus considerable, with the highest recorded value being 3·8× the median and an anticipated 95 per cent lower limit value of about 0·2× the median. Part of this variation must be due to the small numbers of instances at many centres, but most of it must be due to other factors.

The values of $\delta i/\sqrt{\sum i}$ are also given in Table I. In this case there were four hospitals with no recorded instance of joint sepsis, so that the value is indeterminate. The values for the other 15 are shown as a cumulative percentage distribution in Figure 5. The distribution is extremely close to that for the Poisson variance alone and there is therefore no evidence of any variation between the hospitals in their response to the use of an ultraclean air system. In

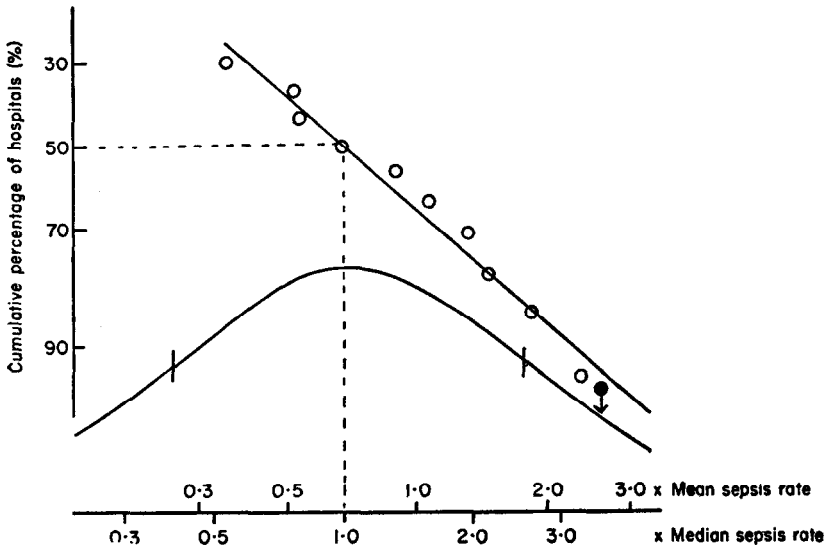

Figure 4. Distribution of the relative risk of joint sepsis at the different hospitals. The upper line shows the distribution of values for the eleven hospitals (○); the extreme upper value is given by a filled circle (●). The lower curve shows the frequency distribution corresponding to the upper cumulative distribution given by the straight line. The 50 per cent medians are shown on both distributions by the broken lines and the range of ± one standard deviation is indicated on the lower curve by short vertical lines. The horizontal scale is given in fractions and multiples of the median value of the sepsis rate (lower scale) and of the arithmetic mean value of this (upper scale).

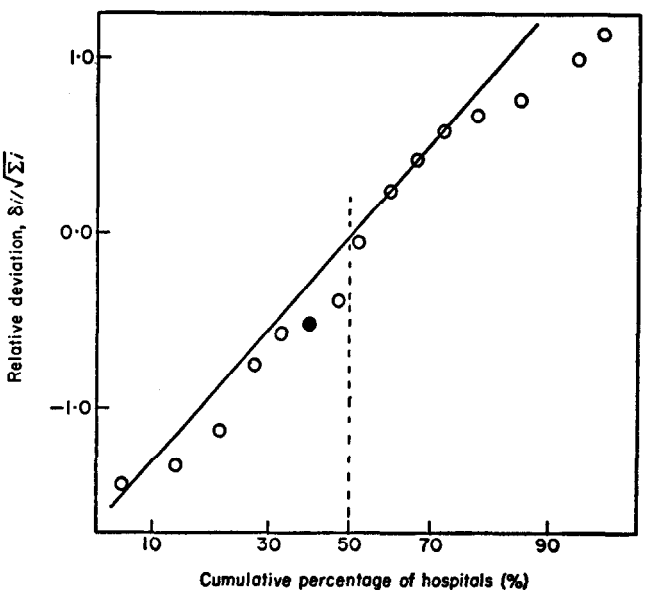

Figure 5. Comparison between the variations in the effect of ultraclean air on sepsis at the different hospitals and that to be expected from the Poisson variance. The line is that for a distribution due to Poisson variance only; the filled circle (●) shows the value for the hospital with the highest value for the relative risk of sepsis (see Figure 4).

particular the hospital with the highest value for the relative risk R is close to the median with regard to the value of $\delta i/\sqrt{\sum i}$.

Discussion

The most interesting conclusion that can be drawn from the data presented in this paper is that it is possible to fit all the results from the several sections of the study into a consistent pattern. The numerical values are subject to considerable possible error because of the relatively small numbers of instances of joint sepsis on which the calculations are necessarily based.

The reduction in the incidence of sepsis as the presumed dose to the wound is decreased is considerably less than proportional. This is in agreement with other observations on the relationship between bacterial dose and the incidence of infection (Lidwell, 1981). However, the lack of proportionality cannot persist down to an indefinitely low dose because this would result in the numbers of those infected eventually exceeding the numbers exposed to even a single bacterial cell. But, within the range of observation, the benefit gained by reducing the level of air contamination is not as great as the percentage reduction in this.

The wide variation in the levels of air contamination at the different hospitals with similar ventilation systems, especially noticeable when conventional pattern clothing was worn in the turbulently ventilated 'control' operating rooms, is conspicuous. Although the difference in the rate of sepsis likely to be a result of those variations is much less than the range of the variations themselves (see, for example, Figure 1), it is by no means insignificant. By limiting the number of persons present in the operating room and by avoiding unnecessary activity it is possible to reduce bacterial dispersal significantly. Increasing the volume of ventilating air will also reduce the resulting level of air contamination. These measures together should avoid the highest values but only an ultraclean air system can provide such clean atmospheres as will substantially reduce the risk of joint sepsis.

The substantial reduction in air contamination levels, and in the incidence of joint sepsis, associated with the use of body-exhaust suits suggests that considerable advantages would result from the development of operating room garments as, or nearly as, effective as these in restricting bacterial dispersal but without the mechanical and tolerability disadvantages. The use of close-woven 'ventile' type cotton fabrics for this purpose has had only limited success in view of the discomfort of an effective, unventilated, suit of this material. Some newer non-woven materials display the barrier effectiveness of these close-woven cotton fabrics while retaining sufficient air porosity for comfort, and the results of preliminary operating room studies are promising (W. Whyte, unpublished data).

The large variation in the relative risk of sepsis at the different hospitals, a range probably at least 20-fold, is not unexpected, but emphasizes the difficulties of drawing conclusions from small numbers of observations at a single centre. The variations might reflect differences in the population at risk or differences in surgical skill and control of operating room conditions. Considerable secular

variations in hospital infection have, however, often been reported, i.e. the irregular occurrence of small 'epidemics' relating to variations in the strains of micro-organisms present in the hospital or the presence of actively dispersing carriers. It is, however, gratifying that these differences do not appear to influence in any substantial way the effect of an ultraclean air environment on the incidence of sepsis, although the absolute value of such a system will, obviously, be potentially greatest where the incidence of sepsis is highest.

Fewer bacteria were isolated from wash-out samples taken during operations done in ultraclean air. These operations also had a lower incidence of joint sepsis. The incidence of sepsis was, therefore, related to the numbers of bacteria in the wash-out samples. The very wide variation in the apparent efficiency of this sampling method at different hospitals makes it difficult to use this approach to explore the effectiveness of control measures in reducing wound contamination until further studies lead to a more reproducible technique. However, it is clear from the results obtained that by far the greater part of bacterial wound contamination in operations for total joint replacement is derived from the air when these are done in a conventionally ventilated operating room.

References

Abel, E. (1973). Measurement of performance, maintenance and indication of correct function of the air supply and extract system. In *Airborne Transmission and Airborne Infection* (Hers, J. F. Ph. and Winkler, K. C., Eds), pp. 519–522. Oosthoek Publishing, Utrecht, Netherlands.

Benediktsdóttir, E. & Hambraeus, A. (1983). Isolation of anaerobic and aerobic bacteria from clean surgical wounds. *Journal of Hospital Infection* **4**, 141–148.

Blowers, R. (1963). Operating room practice. In *Infection in Hospitals* (Williams, R. E. O. and Shooter, R. A. Eds), p. 199. Blackwell Scientific, Oxford.

Hambraeus, A. & Benediktsdóttir, E. (1980). Airborne non-sporeforming anaerobic bacteria. *Journal of Hygiene, Cambridge* **84**, 181–190.

Hare, R. (1963). Dispersal of staphylococci. In *Infection in Hospitals* (Williams, R. E. O. and Shooter, R. A. Eds), pp. 75–86. Blackwell Scientific, Oxford.

Harmer, M. L., Robson, M. C., Krizek, T. J. & Southwick, W. O. (1975). Quantitative bacterial analysis of comparative wound irrigations. *Annals of Surgery* **181**, 819–822.

Hill, J., Howell, A. & Blowers, R. (1974). Effect of clothing on dispersal of *Staphylococcus aureus* by males and females. *Lancet ii*, 1131–1137.

Lidwell, O. M. (1981). Transfer and acquisition of *Staphylococcus aureus* in hospitals. In *The Staphylococci* (Macdonald, A. & Smith, G., Eds), pp. 175–202. University Press, Aberdeen.

Lidwell, O. M., Richards, I. D. G. & Polakoff, G. (1967). Comparison of three ventilating systems in an operating room. *Journal of Hygiene, Cambridge* **65**, 193–205.

Lidwell, O. M., Lowbury, E. J. L., Whyte, W., Blowers, R., Stanley, S. & Lowe, D. (1982). The effect of ultraclean air in operating rooms on deep sepsis in the joint after total hip or knee replacement: a randomised study. *British Medical Journal* **285**, 10–14.

Lindberg, L. (1979). Operations boxar. *Läkartidningen* **76**, 1075–1078.

Mackintosh, C. A., Lidwell, O. M., Towers, A. G. & Marples, R. R. (1978). The dimensions of skin fragments dispersed into the air during activity. *Journal of Hygiene, Cambridge* **81**, 471–479.

May, K. R. & Pomeroy, N. P. (1973). Bacterial dispersion from the human body. In *Airborne Transmission and Airborne Infection* (Hers, J. F. Ph. & Winkler, K. C., Eds), pp. 426–432. Oosthoek Publishing, Utrecht, Netherlands.

Nelson, J. P. (1977). The operating room environment and its influence on deep wound infection. In *The Hip Society Proceedings*, pp. 129–146. C. V. Mosby, St Louis (tables corrected to true metric values; personal communication, 15 December 1981).

Noble, W. C., Hablema, J. D. F., Furth, R. van, Smith, I. & de Raay, C. (1976). Quantitative studies on the dispersal of skin bacteria into the air. *Journal of Medical Microbiology* **9**, 53–61.

Raahave, D. (1979). *Bacterial Densities in Operation Wounds*. FADL's Forlag Århus, Odense, Denmark.

Trezler, P. C. (1973). An isolation system for the maintenance of aseptic environments, *Lancet i*, 91–93.

Whyte, W., Hodgson, R. & Tinkler, J. (1982). The importance of airborne bacterial contamination of wounds. *Journal of Hospital Infection* **3**, 123–136.

Whyte, W., Vesley, D. & Hodgson, R. (1976). Bacterial dispersion in relation to operating room clothing. *Journal of Hygiene, Cambridge* **76**, 367–378.